Atty Dkt No. 2300-1087

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

Cowgill et al.

Serial No.: 08/477,984 Group Art Unit: 1654

Filing Date: June 7, 1995 Examiner: A. Gupta

Title: METHODS FOR PURIFYING AUTHENTIC IGF FROM YEAST HOSTS

DECLARATION PURSUANT TO 37 CFR §1.131

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

We, Cynthia Cowgill, Luis Juarbe-Osorio, Patricio Riquelme, Glenn Dorin, Christopher M. Bussineau and Robert D. Kudrna, hereby declare as follows:

1. We are the inventors of the above-captioned patent application ("the Application").

2. We understand that the U.S. Patent and Trademark Office has rejected the claims in the Application in an Office Action mailed December 4, 1998, on the basis of U.S. Patent No. 5,712,249, to Halloran, entitled "Use of Insulin-Like Growth Factors I and II for Inhibition of Inflammatory Response" (the Halloran patent"), which was derived from an application that was first filed on September 8, 1994.

3. We submit this Declaration to show that we had developed a protocol for isolating IGF from yeast, and, in particular, had conceived of a process for isolating IGF from *Pichia pastoris*, as claimed, prior to September 8, 1994. Attached are excerpts from

an internal production report which establish this. The dates and certain information on the submitted pages have been redacted. However, the work described on these pages predates September 8, 1994 and was performed in the United States. Subsequent to the development of the procedure detailed in the appended documents, we worked diligently to further develop and optimize the claimed IGF purification process.

4. In particular, prior to September 8, 1994, we developed a strategy for isolating IGF from yeast cells which, as detailed on page 1 of the exhibit, included a first cation exchange step, a refold step, a hydrophobic interaction chromatography step (HIC), an optional second cation exchange step (see explanation further below) and a reverse phase high performance liquid chromatography (RP-HPLC) step. Page 2 of the exhibit includes the heading "SP-650S" which refers to the second cation exchange step. Page 3 of the exhibit shows that we contemplated the second cation exchange step to be optional if *P. pastoris* was used.

5. We declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Cynthia Cowgill — Date

Luis Juarbe-Osorio — x 02JUNE99 Date

Patricio Riquelme — Date

Glenn Dorin — Date

Christopher M. Bussineau — Date

Robert D. Kudrna — Date

REDACTED

**Recovery and purification of IGF-1 from recombinant *Saccharomyces cerevisiae* and *Pichia pastoris* cell free supernatants**

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PROCESS OVERVIEW

1) S-fractogel capture to recover all IGF-1 species, including native, oligomeric, scrambled (misfolded), met-oxidized and glycosylated and eliminate some yeast contaminants.

2) Refolding to generate more native IGF-1 from oligomeric and scrambled species.

3) Concentration/ Diafiltration to eliminate refolding salts and avoid precipitation upon pH drop.

4) PAE (HIC) chromatography to purify native IGF-1, decreasing glycosylated and oligomeric species and substantially reducing yeast contaminants.

5) Diafiltration of PAE pool into SP-650S equilibration buffer to eliminate ammonium sulfate and decrease conductivity.

6) SP-650S chromatography to further decrease the amount of glycosylated species.

7) RP-HPLC (Kromasil) to eliminate met-oxidized, glycosylated and des2-IGF-1 species.

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F. SP-650S

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*If the *P. pastoris* feedstock is used, this step can probably be eliminated because of the feedstock's higher purity.

G. RP-HPLC

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